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Gene expression of flap endonuclease-1 during cell proliferation and differentiation

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Abstract

It has been shown that flap endonuclease-1 (FEN-1), a structure-specific nuclease, acts on the removal of RNA primers during Okazaki fragment maturation in DNA synthesis. To study whether the gene expression of FEN-1 is inducible during cell proliferation, we analyzed the FEN-1 mRNA levels in actively growing cells and non-growing cells. The gene expression of FEN-1 was higher in mitotic cells than in resting cells, and was markedly decreased, especially, when terminal differentiation was induced in promyelocytic leukemia cells (HL-60 cells). The decline correlated substantially with the ceasing of DNA synthesis. In the examination of tissue-specific gene expression, the human testis, spleen, thymus and mucosal lining of colon tissues expressed this gene actively, whereas the prostate, ovary, small intestine and peripheral blood leukocyte hardly expressed it. In addition, FEN-1 was co-localized with the proliferating cell nuclear antigen (PCNA) in young rat kidney according to immunohistochemistry. These findings suggest that FEN-1 gene expression is inducible during cell proliferation for DNA synthesis, and is down-regulated during cell differentiation.

Keywords: Cell proliferation; Differentiation; Flap endonuclease-1; Gene expression; Proliferating cell nuclear antigen

1. Introduction

Flap endonuclease-1 (FEN-1) is a structure-specific nuclease which recognizes 5′-branched flap DNA and removes the unannealed single-stranded region [1]. It also exhibits 5′→3′ exonuclease activity with a specificity for double-stranded DNA containing 5′-recessed ends [1]. Mammalian FEN-1 was cloned from murine [2] and human cells [3–4]. On the basis of its nuclease activity and deduced amino acid sequence, it has been understood that FEN-1 is the same enzyme as the maturation factor I (MF I) [4], which acts in SV40 DNA replication in vitro [5]. Because calf 5′→3′ exonuclease and yeast RTH1 also have the same enzyme activity and specificity for DNA substrates as murine FEN-1 [6–7], they are regarded as calf and yeast FEN-1 homolog, respectively. In addition, several archaebacterial FEN-1s were newly cloned and their recombinant proteins

Abbreviations: DMSO, dimethyl sulfoxide; FEN-1, flap endonuclease-1; PCNA, proliferating cell nuclear antigen; PHA, phytohemagglutinin; RA, all-*trans* retinoic acid; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate

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were characterized extensively [8–9]. Therefore, it seems that FEN-1 gene is highly conserved from prokaryotes to eukaryotes.

It has been demonstrated that the calf FEN-1 homolog acts on the removal of RNA primer with its exo- or endonuclease activity during Okazaki fragment processing in a lagging strand DNA synthesis [10–13]. The FEN-1 can also interact with the proliferating cell nuclear antigen (PCNA) [14–16], which is a auxiliary factor for DNA polymerases δ and ε. These facts suggest that the PCNA, with its ring-like structure, loads onto a double stranded DNA by encircling it, and then physically interacts with FEN-1 in order to recruit FEN-1 at the DNA replication fork.

According to a mapping study on the binding site, PCNA binds to FEN-1 at the region at which PCNA binds to $p21^{Cip1}$ [15–16]. It has been understood that one of the important antiproliferative roles of $p21^{Cip1}$ is the inhibition of DNA replication by PCNA–$p21^{Cip1}$ binding. Presumably, the $p21^{Cip1}$ blocks cell proliferation through inhibition of PCNA's role in recruiting FEN-1 as well as DNA polymerases at DNA replication sites.

Using an in vitro system, recent studies have demonstrated that the FEN-1 plays an important role in DNA replication [10–12]. To date, however, little is known about the regulation of this gene expression in cells. We have studied on FEN-1 gene expression, and found that the gene expression was down-regulated in differentiated promyelocytic leukemia cells [17]. The down-regulation was not specific to differentiation-lineage (granulocytic or monocytic), because all of the mRNA levels were decreased in cases of granulocytic differentiation by all-*trans* retinoic acid (RA) or dimethyl sulfoxide (DMSO), and monocytic differentiation by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). It is likely that this gene expression can be blocked in the non-growing cells in which DNA replication is no longer needed. Therefore, we further studied in order to confirm a relationship between FEN-1 gene expression and DNA synthesis. In this study, FEN-1 gene expression was analyzed during cell proliferation, in which DNA synthesis progresses actively, and during cell differentiation, in which it is blocked entirely. The tissue-specific human FEN-1 gene expression was also investigated.

2. Materials and methods

2.1. Cells and cell differentiation

Peripheral blood granulocytes and lymphocytes were separated from healthy volunteers' blood by Ficoll-Hypaque centrifugation as described in [18]. The lymphocytes were activated by 100 μg/ml of phytohemagglutinin P (PHA) in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% FCS (HyClone, Logan, UT).

The HL-60 promyelocytic leukemia cell line was obtained from the American Type Culture Collection (Rockville, MD), and maintained in RPMI 1640 medium containing 10% FCS. To induce cell differentiation, the HL-60 cells were plated at a density of $1–2\times10^5$/ml in a medium containing 1.3% DMSO (Sigma), 1 μM RA (Sigma) or 100 nM TPA (Sigma), and then cultured for 5–7 days. The cell differentiation was evaluated by morphological changes in Giemsa staining [19] and the reduction of c-myc gene expression [20].

2.2. In vivo DNA synthesis

To check DNA synthesis during differentiation, $1–2\times10^5$/ml of HL-60 cells were seeded in 24-well plates (Corning, Corning, NY), cultured with or without differentiating inducers, and incubated with [^{3}H]thymidine (NEN, Boston, MA) at 10 μCi/ml for 2 h before harvesting. The labeled cells were washed with PBS twice, resuspended in 1 ml of cold 10% TCA (Sigma), and then stood on ice for 20 min. The precipitates were collected on Whatman GF/A filters (Whatman, Maidstone, UK). After washing the filters with cold 10% TCA twice, radioactivity was measured using a liquid scintillation counter (Beckman LS 6500).

2.3. Preparation of anti-FEN-1 antibody

The DE3 cells (*E. coli* strain BL21), which were transformed by pET-FCH plasmid containing human FEN-1 cDNA, were obtained from Dr. Min S. Park, Los Alamos National Laboratory (Los Alamos, NM). The FEN-1 protein, which was overexpressed in DE3 cells, was purified by affinity column chromatography under non-denaturing conditions

using Ni^{2+}-Sepharose (Invitrogen, Carlsbad, CA). The antibody was then produced by immunizing rabbits with the purified recombinant human FEN-1, followed by isolating the IgG fraction using the ImmunoPure IgG Purification Kit (Pierce, Rockford, IL) according to the manufacturer's directions.

2.4. Immunohistochemistry

Five-day-old Sprague–Dawley rats were deeply anesthetized with 4% chloral hydrate (1 ml/100 mg) and perfused transcardially with PBS, followed by 4% paraformaldehyde (Sigma) in 0.1 M phosphate buffer (pH 7.2). Kidneys were dissected and postfixed in the same fixative for 4 h at 4°C. After being rinsed in PBS, tissue was dehydrated in a graded series of ethanol and embedded in wax (polyethylene glycerol 400 disterate; Polysciences, Warrington, PA). Consecutive transverse sections of 3 μm were immunostained using the avidin–biotin–horseradish peroxidase technique (Vectastain ABC kit; Vector, Burlington, CA). In brief, the sections were dewaxed, rehydrated, and incubated with 3% H_2O_2 for 30 min to eliminate endogenous peroxidase activity. After treatment with blocking serum for 1 h, the consecutive sections were incubated with rabbit polyclonal antibody against FEN-1 (dilution rate 1:10) or mouse monoclonal anti-PCNA antibody (Dako, Denmark, dilution rate 1:100) at 4°C overnight. Sections incubated with antibody blocked with antigen served as controls. The sections were rinsed in PBS, incubated with the biotinylated secondary antibody against rabbit IgG (FEN-1) or mouse IgG (PCNA) for 2 h, and subsequently incubated with the Vectastain ABC reagent for 1 h. After being rinsed again with PBS, the sections were incubated with peroxidase substrate solution (diaminobenzidine), and then examined with an Olympus microscope (Olympus, Japan).

2.5. Oligonucleotide primers and RT-PCR

The oligonucleotide primers used in this study were synthesized by Genosys (The Woodlands, TX). Their sequences are: FEN-1 upper primer, 5′-GCCAATCCAGGAATTCCACC-3′; and FEN-1 lower primer, 5′-GATTCGCTCCTCAGAGAACTGCTT-3′. The RT-PCR reactions were carried out using a mRNA Selective PCR Kit (TaKaRa, Shiga, Japan) according to the procedures outlined by the manufacturer. In brief, 5 μg of total RNA extracted from each sample was reverse-transcribed for 30 min at 42°C in 1×PCR buffer containing 5 mM $MgCl_2$, 1 mM dNTP/analog mixture, RNase inhibitor, oligo(dT) primer and 0.1 U/μl AMV reverse transcriptase in a total volume of 50 μl. Then 10 μl of the RT reactant was amplified in a total volume of 50 μl. The amplification was performed for 30 cycles under the following reaction conditions: 5 mM $MgCl_2$, 1 mM dNTP/analog mixture, 0.4 μM primers, 0.1 U/μl *Taq* DNA polymerase; denaturation, 85°C for 1 min; annealing, 55°C for 1 min; extension, 72°C for 1 min. The 10 μl of RT-PCR reaction was then electrophoresed on a 1.5% agarose gel, and stained with EtBr.

2.6. Northern blot analysis

Total cellular RNAs were isolated from the HL-60 cells treated with or without various reagents. Six micrograms of RNA sample was electrophoresed on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The FEN-1 mRNA was analyzed by the DIG chemiluminescent detection method according to the procedure recommended by the manufacturer (Boehringer Mannheim, Mannheim, Germany). The probes, incorporated with DIG-11-dUTP, were prepared by PCR amplification (606–963-bp region of human FEN-1 cDNA and 294–1131-bp region of human β-actin cDNA).

The FEN-1 genes expressed in eight different human tissues were also examined by Northern blot analysis using a nylon membrane blotted poly(A)$^+$ RNA of 2 μg, that was purchased from Clontech (Palo Alto, CA).

3. Results

3.1. FEN-1 gene expression in mitotic cells

To examine whether FEN-1 could be induced during cell proliferation, the FEN-1 mRNA levels in mitotic lymphocytes and immature granulocytes were compared with those in resting lymphocytes and mature granulocytes using a RT-PCR method.

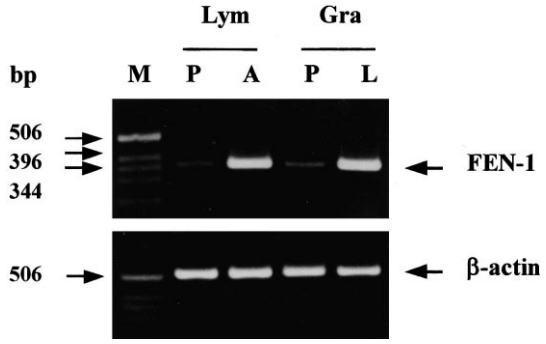

Fig. 1. Up-regulation of FEN-1 gene expression in mitotic cells. Agarose gel electrophoresis patterns of RT-PCR products using total RNA (0.2 μg) extracted from the cells under different growth conditions. Upper and lower panel show amplified 358 bp fragments of FEN-1 gene and 541 bp of β-actin, respectively. Lymphocyte (Lym): peripheral (P), activated with PHA (A); Granulocyte (Gra): peripheral (P), promyelocytic leukemia cells (HL-60) (L).

As shown in Fig. 1, the mRNAs in peripheral blood lymphocytes and granulocytes were detected at low levels. However, the lymphocytes activated with PHA and promyelocytic leukemia cells, which have a much higher proliferating activity than resting lymphocytes and mature granulocytes, respectively, expressed this gene considerably more than their non-growing counterpart cells. The levels of actin mRNA were alike in all tested cells. These observations suggest that the FEN-1 gene expression is up-regulated in actively growing leukocytes.

3.2. Relationship between FEN-1 expression and in vivo DNA synthesis

It has been well known that HL-60 promyelocytic leukemia cells could be differentiated into granulocytes by DMSO or RA, and into monocytes/macrophages by TPA. In order to prepare non-growing cells, terminal differentiation of HL-60 cells was induced by incubating them with 1.3% DMSO or 1 μM RA for 7 days, or with 100 nM TPA for 5 days. After incubation, cell differentiation was evaluated by Giemsa staining [17,19] and observation of the completely blocked c-myc gene expression [20] (data not shown). As shown in Fig. 2A, the fully differentiated HL-60 cells did not synthesize DNA anymore, and also showed a remarkable decline in FEN-1 gene expression (Fig. 2B and [17]). Fig. 3 shows cell growth, DNA synthesis and FEN-1 mRNA levels at different times after RA treatment. The cell growth was retarded during cell differentiation. And the DNA synthesis in RA-treated HL-60 cells was decreased to 36%, 71% and 12% of that in exponentially growing cells (cells cultured for 3 days without RA) after 1, 3 and 5 days, respectively. The change of FEN-1 mRNA levels correlated substan-

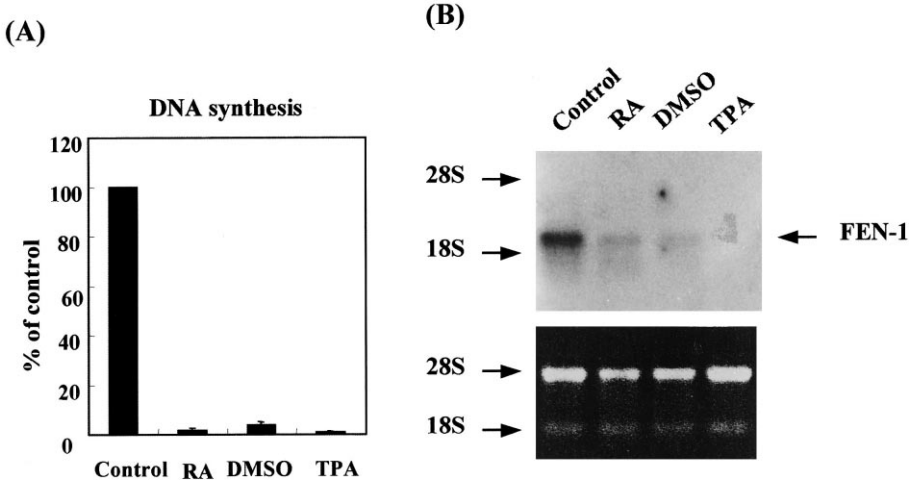

Fig. 2. Down-regulation of FEN-1 expression in differentiated HL-60 cells. HL-60 cells were treated with 1.3% DMSO or 1 μM RA for 7 days, or with 100 nM TPA for 5 days. Control refers to HL-60 cells in exponential growth (cultured for 3 days without inducer). (A) [^{3}H]thymidine incorporation in the fully differentiated HL-60 cells. Results are presented as the mean ± S.D. of triplicate experiments. (B) Total RNA (6 μg) was analyzed with a DIG-labeled PCR product of human FEN-1 cDNA (606–963 bp region) by Northern blot (top). EtBr staining was used for RNA quantity (bottom).

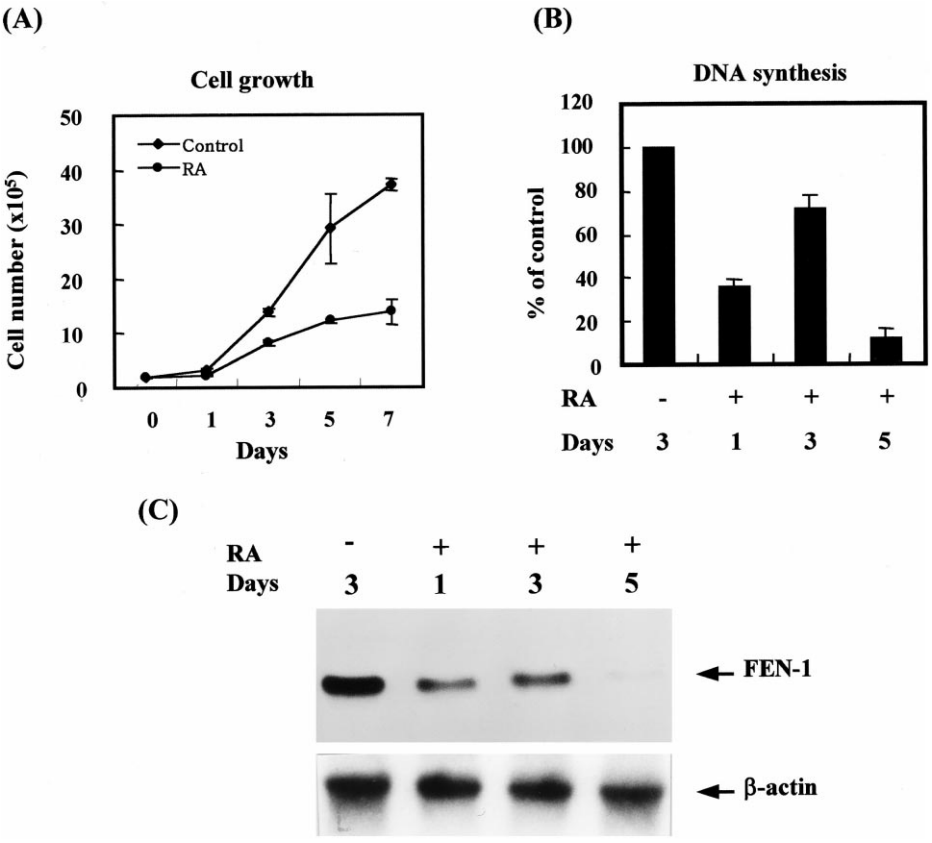

Fig. 3. Time course of DNA synthesis and FEN-1 gene expression during differentiation. (A) The growth curve of HL-60 cells with or without 1 μM RA for 5 days. Cell numbers were counted at the indicated times with a hemocytometer and were expressed as the mean ± S.D. of triplicate experiments. (B) [^{3}H]Thymidine incorporation at various times after RA treatment. Data were given as the mean ± S.D. of triplicate experiments. (C) FEN-1 mRNA level at various times after RA treatment.

tially with that of DNA synthesis, suggesting a positive relationship between in vivo DNA synthesis and FEN-1 gene expression.

3.3. Differential expression of the FEN-1 in young rat kidney

To further confirm the relationship between cell proliferation and the FEN-1 enzyme, localization of FEN-1 protein in rat tissue was observed using immunohistochemistry. First, a PCNA known as DNA replication protein was immunostained using 3-μm consecutive sections of the kidney from a 5-day-old rat, in order to identify the cell proliferating areas in the renal cortex and corticomedullary junction. In the renal cortex, most cells in the collecting ducts were labeled with PCNA, whereas few cells were labeled in the proximal and distal tubules. Several glomeruli did not express PCNA (Fig. 4a). The staining pattern in the corticomedullary junction was similar to that in the cortex (Fig. 4c). Interestingly, FEN-1 could be detected in the area showing PCNA immunoreactivity (Fig. 4b,d), which implies that FEN-1 may participate in DNA replication.

3.4. Tissue-specific human FEN-1 gene expression

Because there have been no previous studies showing the gene expression of FEN-1 in specific human tissues, FEN-1 mRNA was analyzed in various human tissues. A nylon membrane (MTN), to which 2 μg of poly(A)$^+$RNA from eight different human tissues were transferred by Northern blotting, was obtained from Clontech (Clontech, CA). Then the FEN-1 gene transcript in each human tissue was investigated using a DIG-labeled FEN-1 probe (described in Section 2). As the result of hybridization, two bands, 2.3 and 1.8 kb, were detected (Fig. 5).

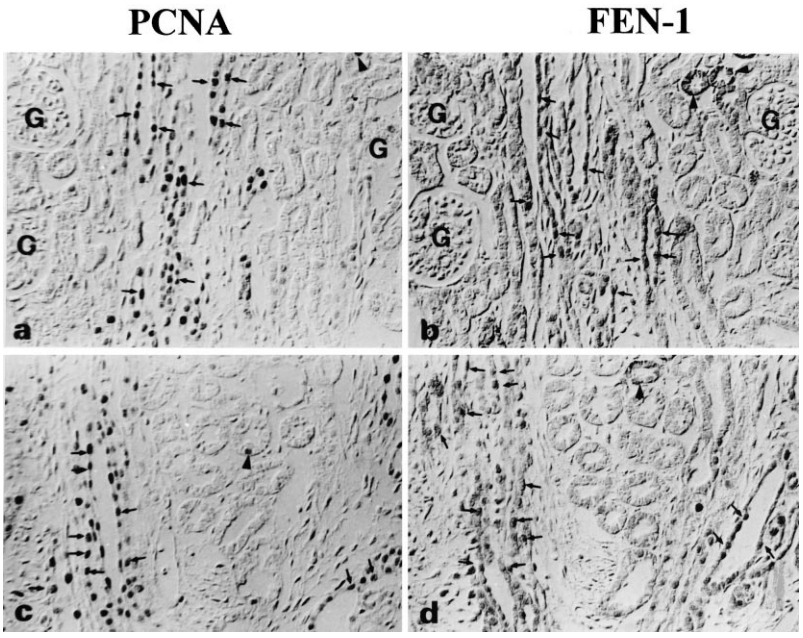

Fig. 4. Light micrographs of immunostaining for PCNA (a,c) and FEN-1 (b,d) in 3-μm consecutive sections of the kidney from 5-day-old rat. (a,b) In the renal cortex, immunostaining for PCNA and FEN-1 appeared simultaneously in the cells of the collecting ducts (arrows). In the proximal and distal tubules, cells labeled for PCNA or FEN-1 were rare (arrowheads). Note that several glomeruli (G) were unlabeled for both antibodies. (c,d) In the corticomedullary junction, most cells of the collecting ducts were labeled with PCNA or FEN-1 (arrows), whereas only a few cells were labeled in the proximal or distal tubules (arrowheads). ×300.

The spleen and testis showed stronger signals than the other tissues, and there were relatively weak signals in the thymus and mucosal lining of the colon. In contrast, the prostate, ovary, small intestine and peripheral blood leukocyte hardly expressed this gene, whereas β-actin expressions were observed very well in these tissues (Fig. 5). These results indicate that the FEN-1 gene is more actively expressed in tissues containing proliferative immature cells.

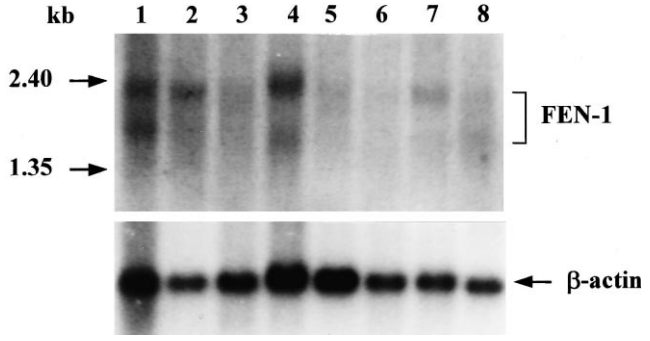

Fig. 5. Tissue-specific human FEN-1 expression. The membrane blotted 2 μg of poly(A)$^+$RNA from each tissue (MTN, Clontech) was hybridized with the specific probes to human FEN-1 (top) and β-actin (bottom). Lanes: 1, spleen; 2, thymus; 3, prostate; 4, testis; 5, ovary; 6, small intestine; 7, colon (mucosal lining); and 8, peripheral blood leukocyte.

4. Discussion

Recent studies have indicated that FEN-1 acts on DNA replication, i.e.: (1) mutant cells lacking in the yeast *RTH1* gene showed deficient cell growth [7]; (2) yeast FEN-1 interacted with a replicative helicase [21] as well as PCNA [22]; and (3) the calf FEN-1 homolog acted in the removal of the RNA primer during Okazaki fragment maturation [11–13]. However, there is no study on the regulation of FEN-1 gene expression. In this study, we investigated FEN-1 expression in cells under different cell-growth stages. The result was that FEN-1 gene expression was up-regulated in the actively growing cells and was markedly down in the non-growing cells. In addition, there was a positive relationship between FEN-1 expression and DNA synthesis. These findings are similar to the results of several other studies indicating that proteins acting in DNA replication,

such as DNA polymerase-α, PCNA and DNA ligase, are inducible during cell proliferation, and down-regulated during cell cycle exit [23–26]. Taken together, it seems likely that FEN-1 expression can be regulated for DNA replication.

When the FEN-1 mRNA was reverse-transcribed and followed by cDNA amplification, only one FEN-1-specific band (358 bp) was observed (Fig. 1). To eliminate an amplified signal originating from contaminated DNA, the mRNA Selective PCR Kit (DaKaRa, Shiga, Japan) was used. However, the possibility of DNA-derived amplification cannot be totally neglected. Actually, a little of the FEN-1 signal showed up in peripheral blood lymphocytes and granulocytes by RT-PCR (Fig. 1), whereas no signal was detected by the Northern blot method (Fig. 5). However, because we were interested in only relative mRNA levels among the cells at the different growth stages, this was not considered. If the amplified mRNA levels were standardized with the β-actin, the FEN-1 expression was always higher in actively proliferating leukocyte cells.

According to our expectations, the induction of terminal differentiation of HL-60 promyelocytic leukemia cells sharply decreased FEN-1 gene expression. This down-regulation was correlated with the cessation of DNA synthesis. The size of mRNA expressed in human leukemia cells was about 2.3 kb (Figs. 2 and 3). This is very close to the transcript size (2.0 kb) of the human FEN-1 gene reported previously [3].

Based on immunohistochemistry, the distribution pattern of FEN-1 enzyme in young rat kidney was similar to that of PCNA. Because it was analyzed using very thin consecutive sections (3 μm), this data could be regarded as co-localization of FEN-1 and PCNA. Recent reports have demonstrated that PCNA interacted with the C-terminus of FEN-1, and that $p21^{Cip1}$ disturbed this PCNA–FEN-1 binding through competition for the binding site on PCNA [15–16]. Therefore, there is a possibility that FEN-1 exists in a complex form with PCNA for DNA replication in the proliferating zones of renal tissues, such as the collecting ducts. Supporting this possibility was the observation that the cells of collecting ducts were not labeled in immunostaining for PCNA if the cells were preincubated with antibody against FEN-1 (data not shown), in contrast, the cells which were not preincubated with anti-FEN-1 antibody were labeled well (Fig. 4a,c).

Two transcripts of the FEN-1 gene, 2.3 and 1.8 kb, were detected in the human spleen and testis (Fig. 5), whereas human leukemia cells have only one, about 2.3 kb (Figs. 2 and 3). We thought that the mRNA of 1.8 kb in normal tissues was a degradated resultant of intact mRNA. Hiraoka et al. reported on two human FEN-1 genes localized in chromosome 1p22.2 and 11q12–q13 that one of these may be functional (11q12–q13), and the other may be a pseudogene (1p22.2) [4]. However, they also did not exclude the possibility that the two genes are both functional. On the basis of this possibility, the 2.3- and 1.8-kb transcripts may be the products of two different genes.

The human *FEN-1* gene (human homolog of *Schizosaccharomyces pombe rad2* gene) restored the UV sensitivity of the yeast *rad2* null mutant up to wild-type levels [3]. In addition, a null mutation in the yeast *RTH1* gene showed an increased instability of the simple repetitive DNA [27] which is associated with human colorectal cancers, and human FEN-1 could efficiently cleave UVDE-incised DNA substrates with photolesions, such as pyrimidine dimers [28]. Therefore, it has been hypothesized that FEN-1 participates in the DNA repair mechanism. Our study did not include the FEN-1 expression during DNA repair. That work is now ongoing.

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References

[1] J.J. Harrington, M.R. Lieber, The characterization of a mammalian DNA structure-specific endonuclease, EMBO J. 13 (1994) 1235–1246.

[2] J.J. Harrington, M.R. Lieber, Functional domains within FEN-1 and RAD2 define a family of structure-specific endonucleases implications for nucleotide excision repair, Genes Dev. 8 (1994) 1344–1355.

[3] J.M. Murray, M. Tavassoli, R. Al-Harithy, K.S. Sheldrick,

A.R. Lehmann, A.M. Carr, F.Z. Watts, Structural and functional conservation of the human homolog of the *Schizosaccharomyces pombe rad2* gene, which is required for chromosome segregation and recovery from DNA damage, Mol. Cell. Biol. 14 (1994) 4878–4888.

[4] L.R. Hiraoka, J.J. Harrington, D.S. Gerhard, M.R. Lieber, C.-L. Hsieh, Sequence of human FEN-1, a structure-specific endonuclease, and chromosomal localization of the gene (*FEN1*) in mouse and human, Genomics 25 (1995) 220–225.

[5] S. Waga, G. Bauer, B. Stillman, Reconstitution of complete SV40 DNA replication with purified replication factors, J. Biol. Chem. 269 (1994) 10923–10934.

[6] R.S. Murante, L. Huang, J.J. Turchi, R.A. Bambara, The calf 5′- to 3′-exonuclease is also an endonuclease with both activities dependent on primers annealed upstream of the point of cleavage, J. Biol. Chem. 269 (1994) 1191–1196.

[7] C.H. Sommers, E.J. Miller, B. Dujon, S. Prakash, L. Prakash, Conditional lethality of null mutations in *RTH1* that encodes the yeast counterpart of a mammalian 5′- to 3′-exonuclease required for lagging strand DNA synthesis in reconstituted systems, J. Biol. Chem. 270 (1995) 4193–4196.

[8] D.J. Hosfield, G. Frank, Y. Weng, J.A. Tainer, B. Shen, Newly discovered archaebacterial flap endonucleases show a structure-specific mechanism for DNA substrate binding and catalysis resembling human flap endonuclease-1, J. Biol. Chem. 273 (1998) 27154–27161.

[9] K.Y. Hwang, K. Baek, H.Y. Kim, Y. Cho, The crystal structure of flap endonuclease-1 from *Methanococcus jannaschii*, Nat. Struct. Biol. 5 (1998) 707–713.

[10] J.J. Turchi, L. Huang, R.S. Murante, Y. Kim, R.A. Bambara, Enzymatic completion of mammalian lagging-strand DNA replication, Proc. Natl. Acad. Sci. USA 91 (1994) 9803–9807.

[11] L. Huang, J.A. Rumbaugh, R.S. Murante, J.R. Lin, L. Rust, R.A. Bambara, Role of calf RTH-1 nuclease in removal of 5′-ribonucleotides during Okazaki fragment processing, Biochemistry 35 (1996) 9266–9277.

[12] R.A. Bambara, R.S. Murante, L.A. Henricksen, Enzymes and reactions at the eukaryotic DNA replication fork, J. Biol. Chem. 272 (1997) 4647–4650.

[13] J.A. Rumbaugh, R.S. Murante, S. Shi, R.A. Bambara, Creation and removal of embedded ribonucleotides in chromosomal DNA during mammalian Okazaki fragment processing, J. Biol. Chem. 272 (1997) 22591–22599.

[14] X. Wu, J. Li, X. Li, C.-L. Hsieh, P.M.J. Burgers, M.R. Lieber, Processing of branched DNA intermediates by a complex of human FEN-1 and PCNA, Nucleic Acids Res. 24 (1996) 2036–2043.

[15] J. Chen, S. Chen, P. Saha, A. Dutta, $p21^{Cip1/Waf1}$ disrupts the recruitment of human Fen1 by proliferating-cell nuclear antigen into the DNA replication complex, Proc. Natl. Acad. Sci. USA 93 (1996) 11597–11602.

[16] E. Warbrick, D.P. Lane, D.M. Glover, L.S. Cox, Homologous regions of Fen1 and $p21^{Cip1}$ compete for binding to the same site on PCNA a potential mechanism to co-ordinate DNA replication and repair, Oncogene 14 (1997) 2313–2321.

[17] I.-S. Kim, Down-regulation of human FEN-1 gene expression during differentiation of promyelocytic leukemia cells, Exp. Mol. Med. 30 (1998) 252–256.

[18] J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober (Eds.), Isolation and Fractionation of Mononuclear Cell Populations and Isolation and Functional Analysis of Neutrophils, In: Current Protocols in Immunology, John Wiley and Sons, 1998, pp. 3.1–3.2 and 7.23.

[19] T.R. Breitman, S.E. Selonick, S.J. Collins, Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid, Proc. Natl. Acad. Sci. USA 77 (1980) 2936–2940.

[20] L.E. Grosso, H.C. Pitot, Modulation of c-myc expression in the HL-60 cell line, Biochem. Biophys. Res. Commun. 119 (1984) 473–480.

[21] M.E. Budd, J.L. Campbell, A yeast replicative helicase, Dna2 helicase, interacts with yeast FEN-1 nuclease in carrying out its essential function, Mol. Cell. Biol. 17 (1997) 2136–2142.

[22] X. Li, J. Li, J. Harrington, M.R. Lieger, P.M.J. Burgers, Lagging strand DNA synthesis at the eukaryotic replication fork involves binding and stimulation of FEN-1 by proliferating cell nuclear antigen, J. Biol. Chem. 270 (1995) 22109–22112.

[23] A.F. Wahl, A.M. Geis, B.H. Spain, S.W. Wong, D. Korn, T.S.-F. Wang, Gene expression of human DNA polymerase α during cell proliferation and the cell cycle, Mol. Cell. Biol. 8 (1988) 5016–5025.

[24] R. Bravo, Synthesis of the nuclear protein cyclin (PCNA) and its relationship with DNA replication, Exp. Cell Res. 163 (1986) 287–293.

[25] L. Montecucco, G. Biamonti, E. Savini, F. Focher, S. Spadari, L. Ciarrocchi, DNA ligase I gene expression during differentiation and cell proliferation, Nucleic Acids Res. 20 (1992) 6209–6214.

[26] A.L. Moore, T.S.-F. Wang, Down-regulation of genes encoding DNA replication proteins during cell cycle exit, Cell Growth Differ. 5 (1994) 485–494.

[27] R.E. Johnson, G.K. Kovvali, L. Prakash, S. Prakash, Requirement of the yeast *RTH1* 5′ to 3′ exonuclease for the stability of simple repetitive DNA, Science 269 (1995) 238–239.

[28] J.-H. Yoon, P.M. Swiderski, B.E. Kaplan, M. Takao, A. Yasui, B. Shen, G.P. Pfeifer, Processing of UV damage in vitro by FEN-1 proteins as part of an alternative DNA excision repair pathway, Biochemistry 38 (1999) 4809–4817.